

WHAT IS CLAIMED IS:

1. A method of synthesizing a target polynucleotide comprising:

- a) providing a target polynucleotide sequence;
- 5 b) identifying at least one initiating polynucleotide present in the target polynucleotide of a), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang and a 3' overhang;
- 10 c) identifying a second polynucleotide present in the target polynucleotide of a), wherein the second polynucleotide is contiguous with the initiating
- 15 polynucleotide and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one
- 20 overhang of the second polynucleotide is complementary to at least one overhang of the initiating polynucleotide;
- 25 d) identifying a third polynucleotide present in the target polynucleotide of a), wherein the third polynucleotide is contiguous with the initiating sequence
- 30 and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the
- 30 third polynucleotide is complementary to at least one overhang of the initiating polynucleotide which is not complementary to an overhang of the second polynucleotide;
- e) contacting the initiating polynucleotide of b) with the second polynucleotide of c) and the third

polynucleotide of d) under conditions and for such time suitable for annealing, the contacting resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;

5 f) in the absence of primer extension, optionally contacting the mixture of e) with a ligase under conditions suitable for ligation; and

 g) optionally repeating b) through f) to sequentially add double-stranded polynucleotides to the
10 extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

2. The method of claim 1, wherein the target polynucleotide sequence encodes a target polypeptide.

15 3. The method of claim 2, wherein the target polypeptide is a protein.

4. The method of claim 3, wherein the protein is an enzyme.

5. The method of claim 1, wherein the initiating
20 polynucleotide sequence is identified by a computer program.

6. The method of claim 5, wherein the computer program comprises the following algorithm:

7. The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 15
25 to 1000 nucleotides in length.

8. The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

9. The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

10. The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 15 to 1000 nucleotides in length.

11. The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

12. The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

13. The method of claim 1, wherein the initiating polynucleotide is attached to a solid support.

14. A method of synthesizing a target polynucleotide comprising:

a) providing a target polynucleotide sequence derived from a model sequence;

b) identifying at least one initiating polynucleotide sequence present in the target polynucleotide sequence of a), wherein the initiating polynucleotide comprises: 1) a first plus strand oligonucleotide; 2) a second plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence that is at least partially complementary to the first plus strand oligonucleotide and second contiguous sequence which is at least partially complementary to the second plus strand oligonucleotide;

c) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide to the minus strand oligonucleotide of b) resulting in a partially double-stranded initiating polynucleotide
5 comprised of a 5' overhang and a 3' overhang;

d) identifying a second polynucleotide sequence present in the target polynucleotide sequence of a), wherein the second polynucleotide sequence is contiguous with the initiating polynucleotide sequence and comprises: 1) a first
10 plus strand oligonucleotide; 2) a second plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence which is at least partially complementary to the first plus strand
15 oligonucleotide and second contiguous sequence which is at least partially complementary to the second plus strand oligonucleotide;

e) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide
20 to the minus strand oligonucleotide of d) resulting in a partially double-stranded second polynucleotide, wherein at least one overhang of the second polynucleotide is complementary to at least one overhang of the initiating polynucleotide;

f) identifying a third polynucleotide present in the target polynucleotide of a), wherein the third polynucleotide is contiguous with the initiating sequence and comprises: 1) a first plus strand oligonucleotide; 2) a
30 plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence which is at least partially complementary to the first plus strand oligonucleotide and second contiguous sequence which is at

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least partially complementary to the second plus strand oligonucleotide;

- g) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide to the minus strand oligonucleotide of f) resulting in a partially double-stranded second polynucleotide, wherein at least one overhang of the third polynucleotide is complementary to at least one overhang of the initiating polynucleotide and not complementary to an overhang of the second polynucleotide;

- h) contacting the initiating polynucleotide of c) with the second polynucleotide of e) and the third polynucleotide of g) under conditions and for such time suitable for annealing, the contacting resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;

i) in the absence of primer extension, optionally contacting the mixture of h) with a ligase under conditions suitable for ligation; and

j) optionally repeating b) through i) to sequentially add double-stranded polynucleotides to the extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

15. A method for synthesizing a target polynucleotide, comprising:

- a) providing a target polynucleotide sequence;
b) identifying at least one initiating polynucleotide present in the target polynucleotide of a), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide;

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annealed fourth oligonucleotide, wherein the extended initiating sequence is extended bi-directionally thereby forming a nascent extended initiating polynucleotide; and

- g) optionally repeating e) through f) as
5 desired, resulting in formation of the target polynucleotide sequence.

16. The method of claim 15, wherein the target polynucleotide sequence encodes a target polypeptide.

17. The method of claim 16, wherein the target
10 polypeptide is a protein.

18. The method of claim 17, wherein the protein is an enzyme.

19. The method of claim 15, wherein the initiating polynucleotide is identified by an algorithm.

- 15 20. A method of synthesizing a target polynucleotide comprising:

- a) providing a target polynucleotide sequence;
b) identifying at least one initiating
polynucleotide present in the target polynucleotide of a),
20 wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of at least a 5' overhang or a 3' overhang;
25 c) identifying a second polynucleotide present in the target polynucleotide of a), wherein the second polynucleotide is contiguous with the initiating polynucleotide and comprises at least one plus strand oligonucleotide annealed to at least one minus strand
30 oligonucleotide resulting in a partially double-stranded

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polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the second polynucleotide is complementary to the overhang of the initiating polynucleotide;

- 5 d) contacting the initiating polynucleotide of
b) with the second polynucleotide of c) under conditions and
for such time suitable for annealing, the contacting
resulting in a contiguous double-stranded polynucleotide,
wherein the initiating sequence is extended uni-
10 directionally;

 e) in the absence of primer extension,
optionally contacting the mixture of e) with a ligase under
conditions suitable for ligation; and

- f) optionally repeating b) through e) to
15 sequentially add double-stranded polynucleotides to the
extended initiating polynucleotide through repeated cycles
of annealing and ligation, whereby a target polynucleotide
is synthesized.

21. The method of claim 15, wherein the plus strand of
20 the initiating, second or third polynucleotide is about 15
to 1000 nucleotides in length.

22. The method of claim 15, wherein the plus strand of
the initiating, second or third polynucleotide is about 20
to 500 nucleotides in length.

23. The method of claim 15, wherein the plus strand of
the initiating, second or third polynucleotide is about 25
to 100 nucleotides in length.

24. The method of claim 15, wherein the minus strand
of the initiating, second or third polynucleotide is about
30 15 to 1000 nucleotides in length.

25. The method of claim 15, wherein the minus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

26. The method of claim 15, wherein the minus strand
5 of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

27. The method of claim 15, wherein the initiating polynucleotide is attached to a solid support.

28. A method for isolating a target polypeptide
10 encoded by a target polynucleotide, comprising:
a) providing a target polynucleotide sequence derived from a model sequence;
b) identifying at least one initiating polynucleotide present in the target polynucleotide of a),
15 wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang and a 3' overhang;
20 c) identifying a second polynucleotide present in the target polynucleotide of a), wherein the second polynucleotide is contiguous with the initiating sequence and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide
25 resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the second polynucleotide is complementary to at least one overhang of the initiating sequence;
30 d) identifying a third polynucleotide present in the target polynucleotide of a), wherein the third polynucleotide is contiguous with the initiating sequence

- and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the third polynucleotide is complementary to at least one overhang of the initiating sequence which is not complementary to an overhang of the second polynucleotide;
- e) contacting the initiating polynucleotide of b) with the second polynucleotide of c) and the third polynucleotide of d) under conditions and for such time suitable for annealing, the contacting resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;
 - f) in the absence of primer extension, optionally contacting the mixture of e) with a ligase under conditions suitable for ligation;
 - g) optionally repeating b) through f) to sequentially add double-stranded polynucleotides to the extended initiating sequence through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized;
 - h) incorporating the target polynucleotide of g) in an expression vector;
 - i) introducing the expression vector of h) into a suitable host cell;
 - j) culturing the cell of i) under conditions and for such time as to promote the expression of the target polypeptide encoded by the target polynucleotide; and
 - k) isolating the target polypeptide.

29. The method of claim 28, wherein the target polypeptide is a chimeric protein.

plurality, wherein the sequence of the plurality of oligonucleotides is a contiguous sequence of the target polynucleotide;

- c) contacting the partially complementary
 - 5 oligonucleotides of b) under conditions and for such time suitable for annealing, the contacting resulting in a plurality of partially double-stranded polynucleotides, wherein each double-stranded polynucleotide is comprised of a 5' overhang and a 3' overhang;
- 10 d) identifying at least one initiating polynucleotide derived from the model sequence, wherein the initiating polynucleotide is present in the plurality of double-stranded polynucleotides set forth in c);
- e) in the absence of primer extension,
 - 15 subjecting a mixture comprising the initiating polynucleotide and 1) a double-stranded polynucleotide that will anneal to the 5' portion of said initiating and sequence; 2) a double-stranded polynucleotide that will anneal to the 3' portion of the initiating polynucleotide;
 - 20 and 3) a DNA ligase under conditions suitable for annealing and ligation, wherein the initiating polynucleotide is extended bi-directionally;
- f) sequentially annealing double-stranded polynucleotides to the extended initiating polynucleotide
 - 25 through repeated cycles of annealing, whereby the target polynucleotide is produced.

40. The method of claim 39, wherein the oligonucleotides are produced by synthesis on an automated DNA synthesizer.

- 30 41. A computer program, stored on a computer-readable medium, for generating a target polynucleotide sequence, the computer program comprising instructions for causing a computer system to:

- a) identify an initiating polynucleotide sequence contained in the target polynucleotide sequence;
- b) parse the target polynucleotide sequence into multiply distinct, partially complementary, oligonucleotides;
- c) control assembly of the target polynucleotide sequence by controlling the bi-directional extension of the initiating polynucleotide sequence by the sequential addition of partially complementary oligonucleotides resulting in a contiguous double-stranded polynucleotide.

42. The computer program of claim 41, wherein the parsing is performed by an algorithm.

43. The computer program of claim 42, wherein the algorithm comprises:

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15  $Overlap = <STDIN>;
    $seqlen = length($sequence);
    }

    $revcomp = "";
    for ($i = $seqlen-1; $i >= 0; $i--)
    {
        $base = substr($sequence,$i,1);
        if ($base eq "a"){ $comp = "T"; }
            elseif ($base eq "t"){ $comp = "A"; }
            elseif ($base eq "g"){ $comp = "C"; }
25      elseif ($base eq "c"){ $comp = "G"; }
            elseif ($base eq "A"){ $comp = "T"; }
            elseif ($base eq "T"){ $comp = "A"; }
            elseif ($base eq "G"){ $comp = "C"; }
            elseif ($base eq "C"){ $comp = "G"; }
30      else { $comp = "X"; }
        $revcomp = $revcomp.$comp;
    }

    print OUT "Forward oligos\n";
35  print "Forward oligos\n";
    $r = 1;
    for ($i = 0; $i <= $seqlen -1; $i+=$OL)
    {
        $oligo = substr($sequence,$i,$OL);
        print OUT "$oligname F- $r      $oligo\n";
40  print "$oligname F- $r      $oligo\n";
        $r = $r + 1;
    }

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    }

    $r = 1;
    for ($i = $seqlen - $Overlap - $OL; $i >= 0; $i--$OL)
5      {
        print OUT "\n";
        print "\n";
        $oligo = substr($revcomp,$i,$OL);
        print OUT "$oligname R- $r    $oligo";
10       print "$oligname R- $r    $oligo";
        $r = $r + 1;
      }
    $oligo = substr($revcomp,1,$Overlap);
    print OUT "$oligo\n";
15    print "$oligo\n";

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wherein

\$oligname is the identifier name for the list and for each component #oligonucleotide;

20 \$OL is the length of each component oligonucleotide;

\$Overlap is the length of the overlap in bases between each forward and each #reverse oligonucleotide;

\$sequence is the DNA sequence in bases;

25 \$seqlen is the length of the DNA sequence in bases;

\$bas is the individual base in a sequence;

\$forseq is the sequence of a forward oligonucleotide;

\$revseq is the sequence of a reverse oligonucleotide;

\$revcomp is the reverse complemented sequence of the gene;

30 \$olignameF[] is the list of parsed forward oligos;

and \$olignameR[] is the list of parsed reverse oligos.

44. The computer program of claim 43, wherein the forward sequence is optionally converted to upper case using

35 an algorithm comprising:

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    $forseq = "";
    for ($j = 0; $j <= seqlen-1; $j++)
    {
        $bas = substr($sequence,$j,1);
        if ($bas eq "a"){ $cfor = "A"; }
40       elseif ($bas eq "t"){ $cfor = "T"; }
        elseif ($bas eq "c"){ $cfor = "C"; }
        elseif ($bas eq "g"){ $cfor = "G"; }
        elseif ($bas eq "A"){ $cfor = "A"; }
        elseif ($bas eq "T"){ $cfor = "T"; }
45       elseif ($bas eq "C"){ $cfor = "C"; }
        elseif ($bas eq "G"){ $cfor = "G"; }
        else { $cfor = "X"; }
    }

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    $forseq = $forseq.$cfor;
    print OUT "$j \n";
  wherein

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5 \$seqlen is the length of the DNA sequence in bases
 \$bas is the individual base in a sequence
 \$forseq is the sequence of a forward
 oligonucleotide.

45. A computer-assisted method for synthesizing a target polynucleotide encoding a target polypeptide derived
 10 from a model sequence using a programmed computer including a processor, an input device, and an output device, comprising:

- a) inputting into the programmed computer, through the input device, data including at least a portion
 15 of the target polynucleotide sequence encoding a target polypeptide;
- b) determining, using the processor, the sequence of at least one initiating polynucleotide present in the target polynucleotide sequence
- 20 c) selecting, using the processor, a model for synthesizing the target polynucleotide sequence based on the position of the initiating sequence in the target polynucleotide sequence using overall sequence parameters necessary for expression of the target polypeptide in a
 25 biological system; and
- d) outputting, to the output device, the results of the at least one determination.

46. The method of claim 45, further comprising predicting, using the processor, whether changing the model
 30 sequence to the target polynucleotide will have an effect on the target polypeptide encoded by the target polynucleotide based on at least one physical, structural or phylogenetic characteristic of the model sequence.

47. A method for automated synthesis of a target polynucleotide sequence, comprising:

- a) providing a user with an opportunity to communicate a desired target polynucleotide sequence;
- 5 b) allowing the user to transmit the desired target polynucleotide sequence to a server;
- c) providing the user with a unique designation;
- d) obtaining the transmitted target polynucleotide sequence provided by the user.

10 48. The method of claim 47, further comprising:

- f) identifying at least one initiating polynucleotide present in the target polynucleotide of e), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang and a 3' overhang;

- g) identifying a second polynucleotide present in the target polynucleotide of e), wherein the second polynucleotide is contiguous with the initiating polynucleotide and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or
- 25 a 5' overhang and a 3' overhang, wherein at least one overhang of the second polynucleotide is complementary to at least one overhang of the initiating polynucleotide;

- h) identifying a third polynucleotide present in the target polynucleotide of e), wherein the third
- 30 polynucleotide is contiguous with the initiating sequence and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide

comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the third polynucleotide is complementary to at least one overhang of the initiating polynucleotide which is not
5 complementary to an overhang of the second polynucleotide;

i) contacting the initiating polynucleotide of f) with the second polynucleotide of g) and the third polynucleotide of h) under conditions and for such time suitable for annealing, the contacting resulting in a
10 contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;

j) in the absence of primer extension, optionally contacting the mixture of i) with a ligase under conditions suitable for ligation; and

k) optionally repeating f) through k) to sequentially add double-stranded polynucleotides to the extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

49. The method of claim 47, further comprising:

f) identifying at least one initiating polynucleotide present in the target polynucleotide of e), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus
20 strand oligonucleotide;

g) contacting the initiating polynucleotide under conditions suitable for primer annealing with a first oligonucleotide having partial complementarity to the 3' portion of the plus strand of the initiating polynucleotide,
30 and a second oligonucleotide having partial complementarity to the 3' portion of the minus strand of the initiating polynucleotide;

h) catalyzing under conditions suitable for primer extension: 1) polynucleotide synthesis from the 3'-hydroxyl of the plus strand of the initiating polynucleotide; 2) polynucleotide synthesis from the 3'-hydroxyl of the annealed first oligonucleotide; 3) polynucleotide synthesis from the 3'-hydroxyl of the minus strand of the initiating polynucleotide; and 4) polynucleotide synthesis from the 3'-hydroxyl of the annealed second oligonucleotide, wherein the initiating sequence is extended bi-directionally thereby forming a nascent extended initiating polynucleotide;

i) contacting the extended initiating polynucleotide of h) under conditions suitable for primer annealing with a third oligonucleotide having partial complementarity to the 3' portion of the plus strand of the extended initiating polynucleotide, and a fourth oligonucleotide having partial complementarity to the 3' portion of the minus strand of the extended initiating polynucleotide;

j) catalyzing under conditions suitable for primer extension: 1) polynucleotide synthesis from the 3'-hydroxyl of the plus strand of the extended initiating polynucleotide; 2) polynucleotide synthesis from the 3'-hydroxyl of the annealed third oligonucleotide; 3) polynucleotide synthesis from the 3'-hydroxyl of the minus strand of the extended initiating polynucleotide; and 4) polynucleotide synthesis from the 3'-hydroxyl of the annealed fourth oligonucleotide, wherein the extended initiating sequence is extended bi-directionally thereby forming a nascent extended initiating polynucleotide; and

k) optionally repeating f) through j) as desired, resulting in formation of the target polynucleotide sequence.

a) providing a user with a mechanism for communicating a model polynucleotide sequence;

c) allowing the user to transmit the model sequence and desired modification to a server;

e) obtaining the transmitted model sequence and desired modification provided by the user;

g) determining, using the processor, the sequence of the model polynucleotide sequence containing the desired modification;

i) selecting, using the processor, a model for synthesizing the modified model polynucleotide sequence based on the position of the initiating sequence in the

51. An isolated polynucleotide composition comprising:

b) a first primer suitable for primer extension having partial complementarity to the 3' portion of the plus strand of the initiating polynucleotide

c) a second primer suitable for primer extension
5 having partial complementarity to the 3' portion of the
minus strand of the initiating polynucleotide; and

d) a solid support matrix,
wherein each of the first and second primers consists of
about 25 to 1000 nucleotides.

10 52. An isolated polynucleotide composition comprising:

a) an initiating polynucleotide comprising a plus strand and a minus strand, wherein the plus or minus strand is modified to incorporate a moiety that binds to a solid support;

15 b) a first primer suitable for primer extension
 having partial complementarity to the 3' portion of the plus
 strand of the initiating polynucleotide

c) a second primer suitable for primer extension having partial complementarity to the 3' portion of the minus strand of the initiating polynucleotide; and

d) a solid support matrix,
wherein each of the first and second primers
consists of about 25 to 1000 nucleotides.